THE HINGE REGION OF IgG3, AN EXTENDED PART OF THE MOLECULE

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1. Introduction

Human IgG is composed of four subclasses: about 70% IgG1, 18% IgG2, 8% IgG3 and 4% IgG4. These subclasses can be distinguished between by their antigenic and chemical properties [1–3]. There are striking subclass differences in biological activities confined to the Fc region. For example, IgG4 does not bind complement, while the other subclasses do and among these IgG3 molecules show the highest binding capacity [4]. In addition, IgG2 does not give a reverse PCA reaction [5] and IgG3 does not react with staphylococal protein A [6]. The only biological activity which so far has been shown to be common to all the IgG subclasses is the capacity for transplacental transfer [7]. In addition to the divergences in biological activities there are also striking differences in sensitivity to various enzymes which have been utilized for subclass typing of myeloma IgG [8] and for isolation of subclass restricted normal IgG and Fc [9]. Both the differences in biological activities and the enzymatic degradation reflect structural differences, of which some have been characterized. For example, the heavy chains are linked by two disulphide bridges in IgG1 and IgG4, by four in IgG2 and five in IgG3 [10, 11]. In many respects IgG3 differs clearly from the other subclasses. For example the heavy chains from the IgG subclasses have a molecular weight of about 50,000, except for the IgG3 heavy chain which has a molecular weight of about 60,000 [12, 13]. Furthermore, when the pepsin digestion product ($F(ab')_2$) of IgG3 is gel filtered it is eluted significantly earlier than the $F(ab')_2$ fragments from the other subclasses [14]. In this study, the structure of the IgG3 heavy chain has been further investigated. Evidence is presented that the extended part of the IgG3 heavy chain is localized to the hinge region where heavy chains are linked together by disulphide bonds. In addition, this part of the $\gamma 3$ chain appears to have an extremely open structure.

2. Materials and methods

Three IgG3 myeloma proteins (Her, Jon, Hus), one IgG1 protein (Ak) and normal IgG were used in this study. The proteins were isolated by DEAE-cellulose chromatography and gel filtration as previously described [15], except for the Her protein which due to a tendency to precipitate at low ionic strength was isolated by elution from a QAE-Sephadex column with 0.015 M phosphate buffer pH 7.6, 0.05 M NaCl. Polypeptide chains were isolated after reduction with 0.2 M 2-mercaptoethanol and alkylation with 0.3 M iodoacetamide followed by gel filtration on Sephadex G-200 with 5 M guanidine–1 M acetic acid as eluant.

Pepsin (Sigma 2X crystallized) was used in an enzyme:substrate ratio of 1:100 and the proteolysis was performed at pH 4.0 for 20 hr. Proteolysis with papain (Sigma 2X crystallized) was carried out at pH 7.0 for 16 hr with an enzyme:substrate ratio 1:100.

All the antisera used were rendered monospecific by absorbing with antigens fixed to CNBr-activated agarose [15]. Gel filtration experiments were always performed using calibrated columns and molecular weight estimates were based on K_{av} values [15, 16].

3. Results

When gel filtered on a calibrated column of Sephadex G-200 at neutral pH, all three IgG3 proteins were eluted with K_{av} 0.13 corresponding to an apparent molecular weight of 190,000, while IgG1 proteins and pooled IgG were eluted with K_{av} 0.19 corresponding to a molecular weight of 150,000 (table 1). After reduction and alkylation of the IgG3 proteins, the heavy chains were eluted on a calibrated column of Sephadex G-200 with 5 M guanidine–1 M acetic acid corresponding to a molecular weight of 60–62,000. IgG1 heavy chains (m.w. 51,000) and light chains (m.w. 22,500) were used as standards. The pepsin digestion product of the three IgG3 myeloma proteins was filtered on a column of Sephadex G-200 in 0.1 M Tris-HCl–0.2 M NaCl–2 mM EDTA pH 7.6 as shown in fig. 1. Material from the main peak (K_{av} 0.19) did not precipitate with anti-Fc antisera but precipitated well with anti-Fab and anti-light chain antisera and was thus assumed to represent $F(ab')_2$ fragments. The K_{av} value of this protein peak corresponded to a molecular weight of 150,000 using IgG, albumin, Fc and Fab from pooled IgG as standards. In control experiments, pepsin digestion of an IgG1 myeloma protein and normal pooled IgG were used, and both gave a main peak with a K_{av} value of 0.25 equal to molecular weight 110,000.

Further experiments were made by dialyzing the $F(ab')_2$ fragments from the IgG3 and IgG1 proteins against 5 M guanidine–1 M acetic acid in order to break non-covalent interaction before gel filtration on a calibrated column of Sephadex G-200 with 5 M guanidine–1 M acetic acid as eluant. Both fragments were eluted in one main peak with K_{av} about 0.10 thus indicating that the early elution position of IgG3 $F(ab')_2$ fragments was due mainly to molecular shape rather than molecular weight.

In order to test this further the $F(ab')_2$ fragments from IgG1 and IgG3 were reduced and alkylated and

Table 1
Molecular weight estimates based on gel filtration.

Material	Column*	K_{av}	Estimated molecular weight
IgG1	1	0.19	150,000
IgG3	1	0.13	190,000
IgG1 $F(ab')_2$	1	0.25	110,000
IgG3 $F(ab')_2$	1	0.19	150,000
IgG1 Fab'	2	0.35	60,000
IgG3 Fab'	2	0.32	69,000
IgG1 Fab	3	0.39	48,000
IgG3 Fab	3	0.42	44,000
IgG1 Fc	3	0.38	52,000
IgG3 Fc	3	0.38	52,000
γ1 chains	4	0.23	51,000
γ3 chains	4	0.18	60,000
IgG1 Fd'	4	~0.45	~ 23,000
IgG3 Fd'	4	0.32	37,000
L-chains	4	0.45	23,000

* Column 1: Sephadex G-200 in Tris-HCl buffer, pH 7.6.
Column 2: Sephadex G-150 in Tris-HCl buffer, pH 7.6.
Column 3: Sephadex G-150 in Tris-HCl buffer, pH 7.6.
Column 4: Sephadex G-200 in 5 M guanidine–1 M acetic acid.

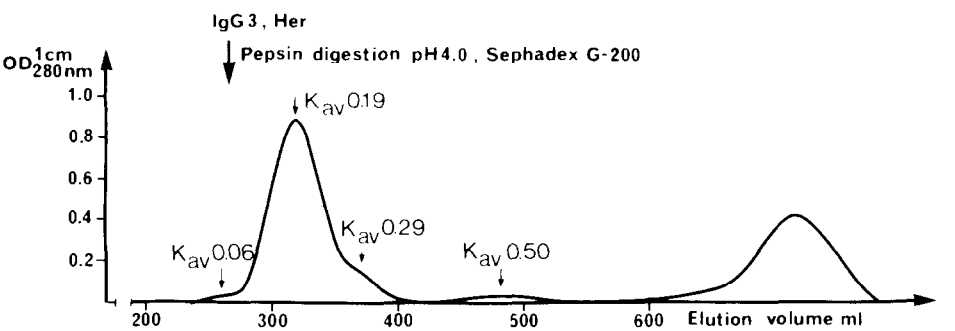

Fig. 1. Gel filtration on Sephadex G-200 of the pepsin digestion product of IgG3 (Her) myeloma protein. Column size: 3.2 × 92.6 cm, v_0 = 223 ml, sample vol: 3.7 ml. Sample conc.: 27 mg/ml. Elution rate: 16.0 ml/hr. Eluant: 0.1 M Tris-HCl–0.2 M NaCl–2 mM EDTA (disodium salt) containing 0.02% sodium azide pH 7.6. Temperature: 23°.

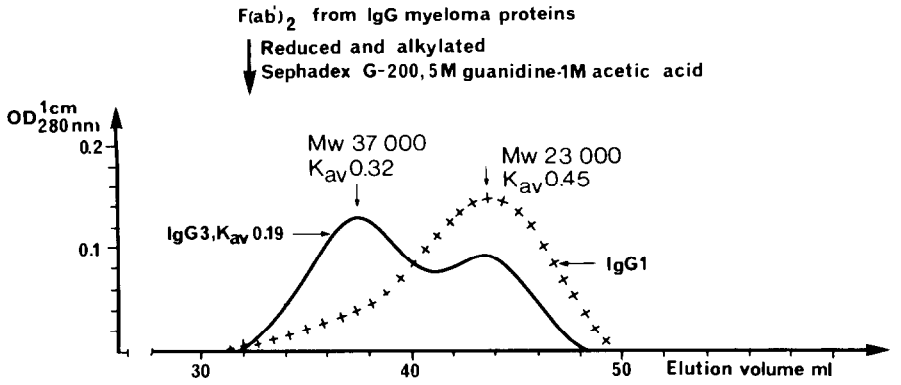

Fig. 2. Gel filtration on Sephadex G-200 of reduced and alkylated F(ab')$_2$ fragments from an IgG3 myeloma protein (Her) and IgG1 myeloma protein (Ak). Column size: 1.5 × 40.1 cm, v_0 = 21.5 ml. Sample volume: 0.4 ml. Sample concentration: 5–10 mg/ml, Elution rate: 1.52 ml/hr. Eluant: 5 M guanidine–1 M acetic acid. Temperature: 23°.

submitted to gel filtration on the Sephadex G-200 column equilibrated with 5 M guanidine–1 M acetic acid (fig. 2). The IgG3 F(ab')$_2$ fragments gave two peaks eluted corresponding to molecular weights of 37,000 and 23,000, respectively (table 1). Using specific anti-Fd and anti-light chain antisera in double immunodiffusion these fragments were identified as Fd' (first peak) and light chains (second peak), respectively. In contrast the IgG1 F(ab')$_2$ fragments gave only one main peak corresponding to a molecular weight of 23,000 when gel filtered after reduction and alkylation, indicating that the IgG1 Fd' has a molecular weight close to 23,000.

The F(ab')$_2$ fragments from IgG3, from IgG1 and from pooled normal IgG were also exposed to mild reduction and alkylation in order to obtain Fab' monomer fragments. The IgG3 Fab' monomer appearing after this treatment was eluted from a calibrated Sephadex G-150 column with 0.1 M Tris-HCl–0.2 M NaCl–2 mM EDTA-Na_2 pH 7.6 corresponding to an apparent molecular weight of 69,000 whilst the Fab' monomer of IgG1 and normal pooled IgG were eluted at a position corresponding to a molecular weight of 60,000. The corresponding Fab fragments were isolated after papain treatment without cysteine by DEAE-cellulose chromatography [9] and the molecular weight calculated according to the K_{av} values on a calibrated column of Sephadex G-150 in Tris buffer, pH 7.6. The molecular weights were 48,000 for the IgG1 and 44,000 for the IgG3 Fab. The Fc from IgG3 was estimated at 52,000 based on similar gel filtration data. The K_{av} values and corresponding molecular weights are summarized in table 1.

4. Discussion

The IgG3 heavy chain has a molecular weight of about 60,000 as reported here and by other groups [13, 17]. This can only partly explain the apparent molecular weight of about 190,000 of intact IgG3 molecules as determined by gel filtration. The F(ab')$_2$ fragments of IgG3 were eluted from gel filtration columns at neutral pH corresponding to a molecular weight of 150,000 while the Fc fragment of IgG3 was eluted corresponding to a molecular weight of 52,000 (table 1). This indicates that the unusual molecular properties of the IgG3 subclass are localized to the F(ab')$_2$ fragment of the molecule. Similar early positions of IgG3 F(ab')$_2$ have recently been reported by Turner et al. [14]. The molecular weight of the IgG3 Fd' was estimated at 37,000 when gel filtered in 5 M guanidine–1 M acetic acid. Thus the two Fd' and two light chains would make up a true molecular weight of 120,000 for the IgG3 F(ab')$_2$. On the other hand, Fd' fragments from IgG1 were eluted under the same conditions together with the light chains, and gave a molecular weight close to 23,000, indicating a true molecular weight of the IgG1 F(ab')$_2$ of about 95,000. As Sephadex G-200 used in 5 M guanidine–1 M acetic

acid can only discriminate poorly between molecular weights above 90,000, this might explain why the IgG1 $F(ab')_2$ and IgG3 $F(ab')_2$ were eluted with the same K_{av} value under these conditions.

The difference between IgG1 Fd′ and IgG3 Fd′ may not be attributable only to a difference in the site of peptic cleavage since the molecular weight of the γ3 chain was estimated at 60,000 and γ1 chain at 51,000. In contrast to IgG3 Fd′, the IgG3 Fd obtained from papain digestion is probably not extended since the molecular weight of the IgG3 Fab fragment was estimated at 44,000, which is slightly lower than that for IgG1 Fab. These data suggest the existence of an insert in the γ3 heavy chain with molecular weight about 10,000 localized to the hinge region and making up the whole difference between the γ3 and γ1 chains. This could account for about 20,000 of the total difference of 40–50,000 between molecular weights of IgG3 and IgG1 and between molecular weights of IgG3 $F(ab')_2$ and IgG1 $F(ab')_2$. The additional discrepancy (20–30,000) probably results from a relatively open conformation within the hinge region in the γ3 chain. This is also partly manifest in the isolated IgG3 Fab′ monomer estimated at 69,000 compared with 60,000 for IgG1 Fab′. The extended hinge region in the IgG3 molecules might explain the great susceptibility of this subclass to proteolysis [18, 19] and their rapid *in vivo* catabolism [20, 21] and may be related to the high number of disulphide bridges and proline residues in the hinge region of this subclass [11]. We have evidence (data to be published) that the open conformation in the hinge is dependent at least partly on intact inter-heavy chain disulphide bridges.

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